

# Use of a minimally invasive method of measuring leaf stomatal conductance to examine stomatal responses to water vapor pressure difference under field conditions

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## Abstract

Most methods of measuring the diffusive conductance to water vapor of individual plant leaves potentially change the leaf environment by enclosing part of the leaf in order to measure the rate of water vapor exchange and the driving force for that exchange. There have been questions about whether leaf to air water vapor pressure difference varies sufficiently in extensive crop canopies to be a significant source of variation in stomatal conductance. Here I combined measurements of temperatures of leaves and metal replicas of leaves with the same size and orientation in an energy balance approach to estimate stomatal and boundary layer conductances under field conditions. The method provides a new way to simultaneously determine the in situ environmental conditions and conductances of leaves. Stomatal conductances of upper canopy leaves of three crop species, *Glycine max*, *Phaseolus vulgaris* and *Zea mays* measured with this method were highly correlated with measurements obtained with a steady-state portable photosynthesis system. However, the photosynthesis system often reduced the leaf to air water vapor pressure difference during the measurements sufficiently to increase stomatal conductance. Measurements made with the minimally invasive method indicated that even in extensive maize and soybean canopies, day-to-day variation in the leaf to air water vapor pressure difference at the surface of upper canopy leaves was sufficiently large to cause about a two-fold variation in mean stomatal conductance on different mid-summer afternoons in both species.

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## 1. Introduction

Idso (1987, 1990) and Idso et al. (1988) questioned whether responses of stomatal conductance to leaf to air water vapor pressure differences ( $D$ ) apparent in measurements made with a porometer actually occurred in leaves in the canopy environment, although the interpretation of Idso et al. has been debated (Monteith,

1990), as discussed in McNaughton and Jarvis (1991). Certainly there are ways in which stomatal conductance measurements can produce a spurious negative correlation between conductance and  $D$ . One example of this is an open gas exchange system in which the water vapor content of the reference air stream, flow rate, and leaf area are constant and stomatal conductance is calculated from the increase in water content of air passing through a cuvette containing leaf material. In such a case, leaf-to-leaf variation in stomatal conductance, whatever the cause, is necessarily highly negatively correlated with  $D$ , especially if leaf temperature is not held constant.

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Grantz and Meinzer (1990) and McNaughton and Jarvis (1991) noted that the water vapor content of air near leaves within canopies may be much higher than that of air above the canopy, particularly for extensive canopies which are poorly coupled aerodynamically to the atmosphere. It is possible that leaf to air water vapor pressure differences at the leaf surface ( $D_0$ ) for leaves within such canopies seldom become large enough to cause stomatal closure.

While precise measurements of stomatal conductance can be made with steady-state and transient porometers and with portable photosynthesis systems, these measurement systems potentially alter the environment by enclosing part or all of a leaf and by altering the air speed over the leaf. Some porometers rely on the assumption that changes in stomatal conductance lag the measurement interval. While this may be valid, in none of these measurement systems is the in situ leaf environment determined. One approach to this problem is to measure leaf temperatures and the air water vapor content independently of the stomatal conductance measurement, and measure stomatal conductance rapidly enough that it does not have time to react to the altered environment imposed during the measurement (e.g. Bunce, 2003). However, that approach does not give information on the in situ leaf boundary layer conductance, and stomatal conductance responds to the water vapor pressure of the air at the leaf surface rather than outside the leaf boundary layer (Bunce, 1985). Determining the water vapor pressure of air at the leaf surface requires knowledge of the boundary layer conductance. Non-invasive measurements of water vapor exchange, such as Bowen-ratio and eddy covariance exist for plant canopies. However, these methods do not directly provide information at the single leaf level, as water vapor flux comes from all leaves in the canopy and from the soil. I developed a method of rapidly determining stomatal and boundary layer conductances of individual leaves under a restricted set of field conditions, where the only interference with the leaf is holding a small thermocouple against the lower leaf surface.

There is a long history of using the energy balance of leaf replicas to estimate leaf boundary layer conductances (reviewed in Brenner and Jarvis, 1995). However, using an energy balance approach to estimate both boundary layer and stomatal conductance is uncommon, because of the difficulty of determining the radiation balance terms for the leaf. I hypothesized that under clear sky conditions, the radiation balance terms for an unshaded horizontal leaf and a metal replica of a leaf above a uniform canopy could be

estimated with sufficient accuracy to provide close estimates of boundary layer and stomatal conductances. While these conditions are restrictive, the new, minimally invasive measurement system could be used to determine the range of  $D_0$  values for extensive maize and soybean crops in Beltsville, Maryland, and to determine whether mid-afternoon  $D_0$  values varied enough from day to day to substantially affect stomatal conductance.

## 2. Methods and materials

The method requires steady-state measurements of leaf temperature, the temperature and water vapor content of the air stream passing over the leaf, the solar radiation incident on the leaf, and the temperature of a metal replica of the leaf held in the same orientation as the leaf and in the same air stream as the leaf. With steady-state conditions, energy balances sum to zero, so:

$$R_{\text{abs}} - L_{\text{oe}} - H - \lambda E = 0 \quad (1)$$

(after Campbell and Norman, 1998, Eq. 14.1), where  $R_{\text{abs}}$  is the absorbed radiation,  $L_{\text{oe}}$  the emitted thermal radiation,  $H$  the sensible heat loss, and  $\lambda E$  is the latent heat loss, which is zero for the leaf replica. The first steps were to determine the radiation balance for the leaf replica ( $R_{\text{abs}} - L_{\text{oe}}$ ), equate that to  $H$ , and use the temperatures of the replica and air to determine the heat conductance ( $g_{\text{Ha}}$ ) from:

$$R_{\text{abs}} - L_{\text{oe}} = H = c_p g_{\text{Ha}} (T_{\text{replica}} - T_{\text{air}}) \quad (2)$$

where  $c_p$  is the molar specific heat of air (after Campbell and Norman, 1998, Eq. 14.1). Estimates of free convection from the leaf replicas indicated that free convection would have been no more than 10% of forced convection in the situations in which I used the instrument. However, Brenner and Jarvis (1995), using heated leaf replicas in an open field, found that during the daytime the assumption that convection was entirely forced was more accurate than assuming a combination of free and forced convection. Therefore I assumed that convection was entirely forced.

The leaf replicas were cut from sheets of soft copper 0.5 mm thick. Thermocouples were soldered in place, a flexible mounting wire attached, and both surfaces then painted with flat black enamel paint. The photosynthetically active radiation incident on the upper surface of the leaf replica was measured with a miniature quantum sensor mounted on the replica parallel to the surface. Temperature of the leaf replica

was measured by a thermocouple on its lower surface about midway between tip and base, and about midway between the center and edge of the replica. Lead wires of the thermocouples were insulated up to the surface of the replica. Replicas of the type of leaf to be measured, with a range of sizes of each shape were constructed. The most suitable replica was chosen on site. For soybeans and beans, replicas matched the shape of terminal leaflets of the trifoliolate leaves. For maize, leaf replicas were rectangular, matching the width of center sections of leaves. The lengths of the rectangles were twice the width.

In use, the replicas were positioned horizontally at the top of canopies. The canopies were assumed to have a reflectivity of 0.20 to short-wave (solar) radiation (a typical value for crops at small (mid-day) zenith angles, from [Campbell and Norman, 1998](#), Table 11.2). The radiation incident on the replicas was estimated from the short-wave radiation on upper and lower surfaces, thermal radiation from the sky on the upper surface, and thermal radiation from the canopy on the lower surface. The short-wave absorptivity of the leaf replicas was set to 0.94, based on measurements of incident and reflected radiation over the 350–970 nm wavelength band, measured with a spectroradiometer (Optronics 740A, Optronics Laboratory, Orlando, FL). Absorptivity of the replicas at longer wavelengths was assumed to be the same as in this wavelength band, and this assumption contributes to uncertainty in the total absorbed short-wave radiation. The short-wave energy incident on the upper surface was calculated from the quantum sensor output, using the conversion factor of 0.39 provided by [LI-COR \(1999\)](#) to convert from photosynthetic photon flux density (in  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) to total energy for sun and sky radiation (in  $\text{W m}^{-2}$ ). [Meek et al. \(1984\)](#) found a higher conversion factor of 0.49, but I used the value from LI-COR since a LI-COR quantum sensor was used to measure photosynthetic photon flux density in this study. The short-wave energy incident on the lower surface was assumed to be 0.20 times that on the upper surface, based on the assumed reflectivity of the canopy below of 0.20, a typical value for crops at small (mid-day) zenith angles. The thermal radiation incident on the upper surface was taken as the clear sky emittance interpolated from [Campbell and Norman \(1998, Table A3\)](#). The thermal radiation incident on the lower surface was taken as the black body radiation for the measured leaf temperature, multiplied by an assumed emissivity of the canopy of 0.97 (a typical value for crop leaves, from [Campbell and Norman, 1998](#), Table 11.3). The small amount of long-wave radiation reflected from the canopy below was

ignored. The long-wave emissivity (and absorptivity) of the leaf replicas was set equal to 0.97 (a typical value, from [Campbell and Norman, 1998](#), Table 11.3). The validity of these assumptions as a whole was tested by comparing the boundary layer conductances of rectangular leaf replicas determined from the energy balance with conductances estimated from wind speed over the replicas. For this test, temperatures of the air and the leaf replica, incident PPFD and horizontal wind speed were determined for leaf replicas held horizontally over grass. Wind speed was measured with a hot-wire anemometer at the same height as the leaf replica, about 4 cm away from it. Height above the grass was varied to produce wind speeds ranging from 0.15 to  $2.0 \text{ m s}^{-1}$ . The boundary layer conductance expected for laminar flow across the leaf replica was compared with that calculated from the energy balance of the leaf replica. Because of the greater turbulence of wind compared with laminar flow, boundary layer conductances in wind are typically about 1.4 times those calculated from laminar flow ([Campbell and Norman, 1998](#)). Boundary layer conductances calculated from energy balance ranged from 1.0 to 2.2 times those predicted from laminar flow, with a mean of 1.4 times, for 10 measurements, with the majority of the measurements falling in the 1.2–1.6 range. This indicates that the estimation of the boundary layer conductance of the leaf replicas by energy balance was reasonably accurate and unbiased. The sensitivity of estimates of boundary layer conductance to the assumptions about emissivity, absorptivity, and reflectivity of radiation, errors in measurements of temperatures and photosynthetic photon flux density, and differences in dimension between leaf replicas and real leaves is presented in [Table 1](#).

Leaves in which stomatal conductance was estimated from energy balance were unshaded, nearly horizontal upper canopy leaves in the same air stream as the leaf replicas. The temperature of the leaf was measured by a thermocouple pressed against the lower surfaces about midway between tip and base, and about midway between the center and edge of the leaf. The thermocouple junction used to measure leaf temperature was mounted on the apex of a wedge of foam insulation which insulated the lead wires up to the leaf surface, and was mounted on a 15 cm long piece of copper wire, which provided enough elasticity to hold the thermocouple against the leaf surface without changing the orientation of the leaf. The incident radiation on the leaf surfaces was assumed to be the same as for the leaf replicas. It was assumed that both leaf surfaces emitted black body radiation at the

Table 1

Sensitivity of estimates of boundary layer conductance to water vapor ( $g_a$ ) of a leaf to errors in assumed values of short-wave absorptivity ( $\alpha$ ) of the replica of the leaf, short-wave reflectivity of the canopy ( $\rho$ ), thermal emissivity ( $\varepsilon$ ) of the replica and leaves, errors in measurement of temperatures of air ( $T_a$ ) and the leaf replica ( $T_r$ ), photosynthetic photon flux density ( $Q_p$ ), and differences in dimension ( $d$ ) between the leaf replica and the leaf

| Variable                                       | Assumed value | Alternative value | Alternative $g_a$ | Change (%) |
|--|---------------|-------------------|-------------------|------------|
| $\varepsilon$                                  | 0.97          | 0.94              | 1.34              | +9         |
| $\alpha$                                       | 0.94          | 0.90              | 1.13              | –8         |
| $\rho$   | 0.20          | 0.15              | 1.15              | –7         |
| $T_a$ (°C)                                     | 31.6          | 31.9              | 1.26              | +2         |
| $T_r$ (°C)                                     | 48.1          | 48.6              | 1.18              | –4         |
| $Q_p$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) | 1790          | 1890              | 1.34              | +9         |
| $d$ (cm)                                       | 6             | 6.6               | 1.17              | –5         |

The original estimate of  $g_a$  for this replica of a soybean leaf was  $1.23 \text{ mol m}^{-2} \text{s}^{-1}$ , which is close to the overall mean value for the observations in this study. Alternative values for each variable are arbitrary estimates of the likely maximum deviation.

measured leaf temperature. The short-wave absorptivity and reflectivity, and long-wave emissivity (and absorptivity) of the leaves were set equal to those of typical crop leaves (from Campbell and Norman, 1998, Tables 11.2, 11.3, 11.4), at 0.50, 0.20 and 0.97, respectively. The leaf was assumed to have the same boundary layer conductance to heat as the leaf replica. For the leaf:

$$R_{\text{abs}} - L_{\text{oe}} - H = \lambda E = \frac{\lambda g_v (e_s(T_{\text{leaf}}) - e_a)}{p_a} \quad (3)$$

where  $\lambda$  is the latent heat of evaporation of water,  $g_v$  the conductance to water vapor,  $e_s(T_{\text{leaf}})$  the saturated vapor pressure at the leaf temperature,  $e_a$  the vapor pressure of air outside the boundary layer, and  $p_a$  is atmospheric pressure (after Campbell and Norman, 1998, Eq. 14.1). Correct calculation of stomatal conductance from  $g_v$  depends on the relative stomatal conductance of the two surfaces (Campbell and Norman, 1998, Eq. 14.2). All three of the species used in this study are amphistomatous, and preliminary measurements with a single surface porometer indicated nearly equal conductances on both surfaces in all species. Hence stomatal conductances were calculated assuming that upper and lower leaf surfaces had equal stomatal conductances. Sensitivity of stomatal conductances to errors in this assumption, errors in measurements of leaf and air temperature, humidity, photosynthetic photon flux density, boundary layer conductance, and assumed values of leaf emissivity, absorptivity and reflectivity are given in Table 2.  $D_0$ , the difference in water vapor

Table 2

Sensitivity of estimates of stomatal conductance to water vapor ( $g_s$ ) of a leaf to errors in assumed values of thermal emissivity ( $\varepsilon$ ), short-wave absorptivity ( $\alpha$ ) and reflectivity ( $\rho$ ) of the leaf, errors in measurement of temperatures of air ( $T_a$ ), the leaf ( $T_l$ ), photosynthetic photon flux density ( $Q_p$ ), and relative humidity (rH), the ratio of adaxial to abaxial stomatal conductance (ratio), and boundary layer conductance to water vapor ( $g_a$ )

| Variable                                       | Assumed value | Alternative value | Alternative $g_s$ | Change (%) |
|--|---------------|-------------------|-------------------|------------|
| $\varepsilon$                                  | 0.97          | 0.94              | 647               | +6         |
| $\alpha$                                       | 0.50          | 0.55              | 719               | +17        |
| $\rho$   | 0.20          | 0.15              | 647               | +6         |
| $T_a$ (°C)                                     | 31.6          | 31.9              | 650               | +6         |
| $T_l$ (°C)                                     | 31.2          | 31.7              | 520               | –15        |
| $Q_p$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) | 1790          | 1890              | 645               | +6         |
| rH (%)   | 51            | 53                | 654               | +7         |
| Ratio  | 1             | 2                 | 642               | +5         |
| $g_a$ ( $\text{mol m}^{-2} \text{s}^{-1}$ )    | 1.23          | 1.35              | 585               | –4         |

The original estimate of  $g_a$  for this replica of a soybean leaf was  $1.23 \text{ mol m}^{-2} \text{s}^{-1}$ , which is close to the overall mean value for the observations in this study, and the original estimate of  $g_s$  was  $612 \text{ mmol m}^{-2} \text{s}^{-1}$ , which is close to the mean value for the maize and soybean leaves in this study. Alternative values for each variable are arbitrary estimates of the likely maximum deviation. Errors in  $\varepsilon$ ,  $\rho$ ,  $T_a$  and  $Q_p$  also affect leaf boundary layer conductance estimates, and those effects are included here.

pressure between air inside the leaf and at the leaf surface, was calculated as transpiration rate divided by stomatal conductance (Bunce, 1985).

The necessary sensors were configured as a hand-held portable “wand”, attached to a console which was carried by a shoulder strap, and used for data display and logging. Air temperature and relative humidity were measured by a miniature thermistor and a capacitance humidity sensor (Humicap sensor, Vaisala, Vantaa, Finland), respectively. These sensors were shaded from direct sunlight, and were ventilated by a small, low capacity fan which produced an air speed of about  $0.2 \text{ m s}^{-1}$ . All temperature sensors matched within the resolution ( $0.1^\circ\text{C}$ ) of the data logger. The “wand” was held so that with the leaf thermocouple pressed against the lower leaf surface, the leaf replica would have the same height and orientation as the leaf but was about 10 cm away from the leaf. The air temperature and relative humidity sensors were positioned to form an equilateral triangle between these sensors, the leaf, and the leaf replica, all in the same horizontal plane. The leaf replica and air temperature sensors were positioned so they were not directly upstream or down-stream of the measured leaf. This required the user to be aware of the wind direction, and to select leaves positioned so that other leaves did not interfere with the sensors. In the three crop species used

here, maize, soybean and bean, suitable leaves occurred at least every few meters of row.

Steady-state conditions required at most a few tens of seconds after the sensors were in place, at least on the clear days I took measurements. The temperature of the leaf replica was the slowest to reach a steady value. The time required for this could be minimized by keeping the replica fully exposed to sunlight and in the approximate orientation of leaves between measurements. After the displayed sensor data appeared steady, a key press on the logger collected sensor values once per second for the next 10 s and stored mean values and ranges of values. Storing information on the variability over time facilitated the editing out of measurements where non-steady conditions occurred.

### 2.1. Plant material

Four varieties of beans (*Phaseolus vulgaris* L.), Jaguar, Matterhorn, Early Gallatin, and Red Hawk were planted in small plots at the South Farm of the Beltsville Agricultural Research Center, Beltsville, Maryland. Three 4 m long rows of each variety were planted in mid-June 2003 with 50 cm between rows, at an overall density of 14 plants  $\text{m}^{-2}$ . Conductance measurements were made in mid-July. Soybean, *Glycine max* L. Merr. cv. S38-T8, and maize, *Zea mays* L. cv. SS885 grown in large plots (about 6 ha each) at the South Farm in 2004 were also utilized. Soybeans and maize were grown with row spacings of 30 and 76 cm, respectively, with overall densities of 25 and 8 plants  $\text{m}^{-2}$ , respectively. Conductance measurements were made on maize from mid-June through mid-July, when plants were 1.25–2 m tall. Soybeans were measured from mid-July through mid-August, when plants were 0.6–1 m tall. There were no significant soil moisture deficits before or during any of the conductance measurements for any of the crops.

### 2.2. Comparisons with a photosynthesis system

Direct comparisons of leaf stomatal conductances measured with the new method and with a portable photosynthesis system were conducted on two dates with the four varieties of bean, and on two other dates each with maize and soybean. These particular measurements on maize and soybean were made near the borders of the large plots, and were not included in the analysis of conductances in extensive canopies. Near noon on clear days, measurements of stomatal conductance of mature, fully illuminated, upper canopy leaves were made on five leaves per plant variety, first with the minimally invasive method, and within 20 min

also with a CIRAS-1 portable photosynthesis system (PP Systems, Amesbury, MA). The CIRAS system used a broad leaf cuvette, enclosing 2.5  $\text{cm}^2$  leaf area, and had automatic  $\text{CO}_2$  and water vapor control. The reference carbon dioxide concentration was adjusted so that the  $[\text{CO}_2]$  external to the sampled leaf area was within 10  $\mu\text{mol mol}^{-1}$  of that of outside air. The reference water vapor concentration was adjusted so that the water vapor pressure of the air external to the leaf boundary layer was within 0.1 kPa of that of outside air. The cuvette had no active temperature control, but used a thermal radiation filter and internal and external ventilation to provide near ambient air temperatures. The boundary layer conductance to water vapor of leaves in the cuvette was 4.3  $\text{mol m}^{-2} \text{s}^{-1}$ . Leaves were left in the cuvette for several minutes, until steady-state conductance values occurred. Different individual leaves were measured with the two methods. Comparisons were made between the mean values of stomatal conductance of the five leaves per group.

### 2.3. Environment and conductance in extensive crop canopies

Measurements of leaf boundary layer and stomatal conductance were made with the minimally invasive method in the large maize and soybean plots in mid-afternoon (14:00–16:00 h) on clear days. Measurements were made on 7 days for maize and 9 days for soybean. Conductances of 10 mature, fully illuminated upper canopy leaves of plants several rows in from the leeward edge of the plots were measured on each occasion, and the fetch was at least 200 m. The temperature and water content of air, and wind speed 2 m above grass at a weather station about 0.5 km from the plots was also determined at the time of the leaf conductance measurements.

## 3. Results and discussion

The comparisons of boundary layer conductance of the leaf replica calculated from energy balance and from wind speed, and the sensitivity analysis (Table 1) support the hypothesis that for the restricted set of conditions used here, radiation balance could be estimated with enough accuracy to allow close estimates of boundary layer conductance under field conditions. Others (e.g. Brenner and Jarvis, 1995) have used pairs of heated and unheated reflective leaf replicas to estimate boundary layer conductance. This has the advantage of simplifying the radiation budget, but because we also needed to determine the leaf radiation



budget in order to estimate leaf stomatal conductance, there was no particular advantage of using that approach to simplify the radiation budget for the leaf replica. Use of a black coating on the leaf replica increased its absorptivity to short-wave radiation to a value high enough that uncertainty in that value did not have a large effect on calculated boundary layer conductance (Table 1). In the species used, it was not difficult to sample leaves with dimensions within 10% of those of the leaf replica. A 10% difference in dimension resulted in about a 5% change in boundary layer conductance (Table 1), and a smaller change in stomatal conductance (Table 2). For larger deviations in dimension, adjustments to boundary layer conductance could be calculated from the fact that conductance is proportional to the square root of the ratio of air velocity to leaf dimension (Campbell and Norman, 1998, Eq. 7.30). The sensitivity of estimates of stomatal conductance to moderate uncertainty in the ratio of abaxial to adaxial stomatal conductances was not large (Table 2). However, the range in that ratio among species is very large. It would be worthwhile determining the approximate ratio, as was done here, and calculating stomatal conductance accordingly.

Stomatal conductances varied by a factor of almost 2 among the four varieties of beans on both measurement dates. Mean stomatal conductances measured with the two methods were highly correlated for the bean data, and for measurements on all three species combined (Fig. 1). The portable photosynthesis system indicated

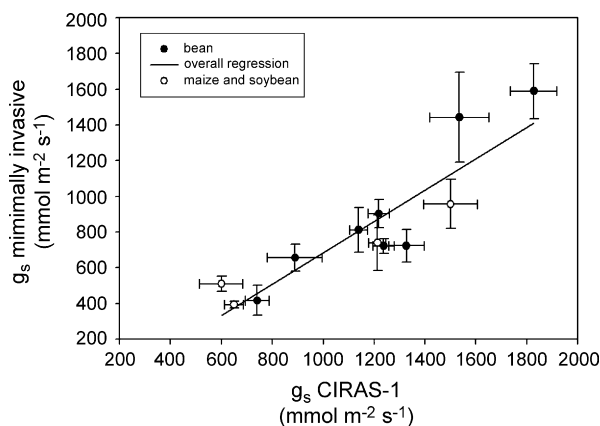
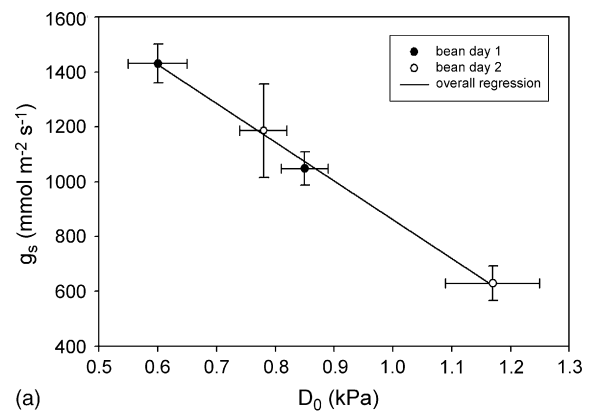
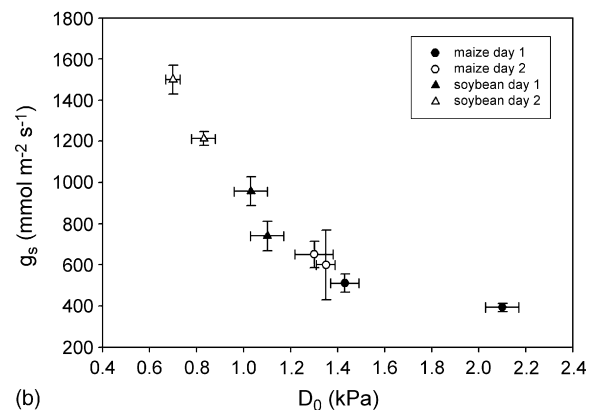


Fig. 1. Stomatal conductance ( $g_s$ ) measured using the minimally invasive method described in this paper and with a CIRAS-1 portable photosynthesis system on four varieties of bean and on maize and soybean. Each species was measured on two dates, and each point represents a mean value for five leaves measured with each method. The overall linear regression equation had an intercept of  $-194 \pm 165$ , a slope of  $0.879 \pm 0.136$ , and an  $r^2$  value of 0.807. Error bars represent S.E., for  $n = 5$ .

higher stomatal conductances, on average by 29%. However, leaf temperatures during the measurements of conductance averaged  $2.4^\circ\text{C}$  lower for measurements made with the portable photosynthesis system than for measurements with the minimally invasive system. This resulted in lower  $D_0$  values in the portable photosynthesis system on each measurement date. In beans, the  $D_0$  values of the measurements made with the two instruments overlapped on the 2 days, and all data defined a single linear relationship between stomatal conductance and  $D_0$  (Fig. 2a). This indicates that the difference in the  $D_0$  between the two methods accounted for the differences in conductance. In maize and soybeans, the  $D_0$  values for the two instruments did not overlap on these particular measurement dates, so it was not as conclusive that  $D_0$  differences between instruments fully accounted for the differences in mean



(a)



(b)

Fig. 2. (a and b) Relationships between stomatal conductance ( $g_s$ ) and the leaf to air water vapor pressure difference of air at the leaf surface ( $D_0$ ), for the data on beans, and maize and soybean in Fig. 1. The two values for a given day are for the two methods of measuring  $g_s$ , with the minimally invasive method always having a greater  $D_0$  than the portable photosynthesis system. Each point represents a mean value for 20 leaves for bean and 5 leaves for maize and soybean. Error bars represent S.E.

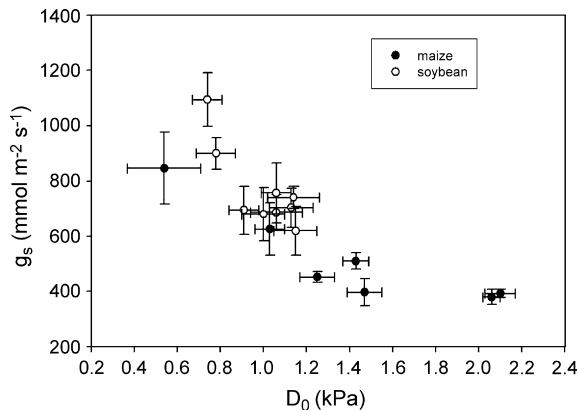


Fig. 3. Relationships between stomatal conductance ( $g_s$ ) and the leaf to air water vapor pressure difference of air at the leaf surface ( $D_0$ ) for maize and soybean leaves measured at mid-afternoon on different days. Measurements were made with the minimally invasive method described in this paper, on the crops grown in large plots. Each point represents a mean value for 10 leaves on a single day. Regression equations and statistics are given in Table 1. Error bars represent S.E.

conductance in these species (Fig. 2b), although other evidence indicates that this was probably the case (see later).

In the measurements on maize and soybeans in the large plots, mean values of stomatal conductance measured with the minimally invasive system on the different dates were correlated with mean  $D_0$  in both species (Fig. 3). There were no significant correlations between either stomatal conductance or  $D_0$  and temperature in either species (not shown). In soybeans, the relationship between stomatal conductance and  $D_0$  obtained with the minimally invasive system was virtually identical to that reported earlier for this species in this environment (Wilson and Bunce, 1997) using a traditional open leaf gas exchange system (Table 3). Also, in maize, the relationship between stomatal conductance and  $D_0$  obtained with the minimally invasive system was very similar to that obtained in an

earlier study (Bunce, 2005) using the same portable photosynthesis system used in this study (Table 3). This is further evidence that the minimally invasive method of measuring stomatal conductance agreed with conventional invasive measurements provided leaves were at the same  $D_0$ . The difficulty in assessing in situ stomatal conductances with conventional methods is therefore in making the measurements at the appropriate  $D_0$  value (for methods slower than the stomatal reaction time) and/or in knowing what that value is (for rapid methods).

Air in the upper part of the canopy of the large maize and soybean plots was cooler and wetter than that 2 m above grass at the weather station on each measurement day. The air in the maize canopy averaged 2.4 °C cooler and 0.48 kPa wetter, while the air in the shorter soybean canopy averaged 1.1 °C cooler and 0.65 kPa wetter. In the measurements in the extensive canopies, the range in air saturation deficits for water vapor at the weather station was 1.2–2.1 kPa on the dates when soybean conductances were measured, compared with the range of  $D_0$  values of 0.74–1.14 kPa (Fig. 3), and air saturation deficits ranged from 1.05 to 2.85 kPa on the dates when maize conductances were measured, compared with  $D_0$  values of 0.54–2.10 kPa (Fig. 3). While the day-to-day range of  $D_0$  values was smaller in soybeans than in maize, the relationship between stomatal conductance and  $D_0$  was steeper in soybean than in maize (Fig. 3), thus giving similar relative day-to-day variation in stomatal conductance in both species. Mean stomatal conductances on the different measurement dates varied by a factor of 2.2 in maize and 1.8 in soybean. Because of the limited number of afternoons sampled, these ranges probably do not represent the maximum ranges of conductance values in this climate, but do indicate that day-to-day variation in  $D_0$  values even in extensive crop canopies is large enough to cause substantial variation in afternoon stomatal conductance in these species.

Table 3

Linear regressions of the natural log of stomatal conductance on the leaf to air water vapor pressure difference at the leaf surface for maize and soybeans, based on day-to-day variation in mid-afternoon values measured with the minimally invasive method described in this paper, on plants grown in large plots, or measured with a portable photosynthesis system (maize) or a conventional open gas exchange system (soybean)

| Species | Method                             | Intercept $\pm$ S.E. | Slope $\pm$ S.E.   | $r^2$ |
|---------|------------------------------------|----------------------|--------------------|-------|
| Soybean | Minimally invasive                 | 7.54 $\pm$ 0.25      | −0.919 $\pm$ 0.240 | 0.669 |
| Soybean | Open system <sup>a</sup>           | 7.39 $\pm$ 0.11      | −0.910 $\pm$ 0.072 | 0.786 |
| Maize   | Minimally invasive                 | 6.89 $\pm$ 0.15      | −0.484 $\pm$ 0.100 | 0.818 |
| Maize   | Photosynthesis system <sup>b</sup> | 7.25 $\pm$ 0.14      | −0.596 $\pm$ 0.093 | 0.767 |

Stomatal conductance is in units of  $\text{mmol mol}^{-2} \text{s}^{-1}$ , and vapor pressure difference is in units of kPa.

<sup>a</sup> After Wilson and Bunce (1997).

<sup>b</sup> Data set described in Bunce (2005).

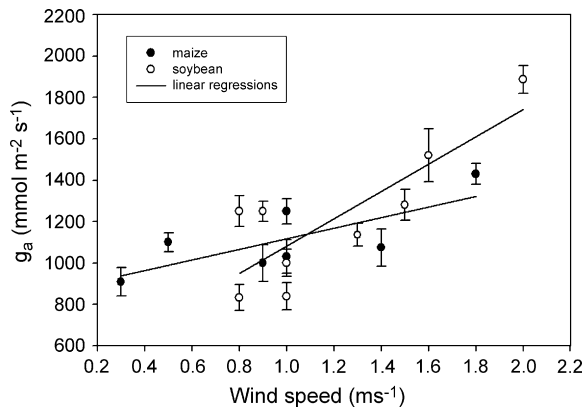


Fig. 4. Relationships between leaf boundary layer conductances to water vapor ( $g_a$ ) of maize and soybean leaves in large plots and the mean wind speed at 2 m above grass. Each point represents a mean value of  $g_a$  of 10 leaves on a given measurement day. Linear regressions are shown for illustration only. Error bars represent S.E.

Mean leaf boundary layer conductances to water vapor ranged from 0.9 to 1.4  $\text{mol m}^{-2} \text{s}^{-1}$  on the 7 measurement dates in maize and from 0.8 to 1.9  $\text{mol m}^{-2} \text{s}^{-1}$  on 9 different measurement dates in soybean. Boundary layer conductances for individual leaf measurements ranged from 0.7 to 2.0  $\text{mol m}^{-2} \text{s}^{-1}$  in maize and from 0.5 to 2.2  $\text{mol m}^{-2} \text{s}^{-1}$  in soybean. In both species, mean leaf boundary layer conductances on a given date increased with increasing mean wind speed measured at 2 m height at the weather station (Fig. 4). Mean wind speeds at 2 m height at the weather station varied from 0.6 to 2.0  $\text{m s}^{-1}$  during the measurements of leaf conductances on the different dates. These are typical mid-afternoon wind speeds in summer at this location. The in situ leaf boundary layer conductances for these species were thus much smaller than occurred in this particular photosynthesis system. Most ventilated porometers and photosynthesis systems have high boundary layer conductances, partly because they enclose small areas of leaves, and partly in order to maximize the sensitivity of measurements of stomatal conductance. Even the use of null-balance systems would not ensure that stomatal conductance would be measured at the in situ  $D_0$  because of possible changes in boundary layer conductance caused by the measurement process.

On a given measurement date, the leaf-to-leaf variation in stomatal conductance measured with the minimally invasive system was sometimes quite large. In maize, the average coefficient of variation of stomatal conductance (i.e. standard deviation/mean) was 0.30. It ranged from 0.11 to 0.49 on different days. The average coefficient of variation of stomatal conductance in

soybeans was 0.33, with a range of 0.17–0.45. Reasons for the large range in coefficients of variation are not known, but there was no correlation between the mean value of conductance and the coefficient of variation in either species. The mean coefficients of variation of stomatal conductance obtained with the minimally invasive system are not substantially different than what I have found with the portable photosynthesis system based on many days of field measurements on these species, 0.26 and 0.29 in maize and soybean, respectively (Bunce, unpublished data).

Significant negative correlations between stomatal conductance and  $D_0$  within a measurement date occurred on only 3 of 9 dates in soybean and 3 of 7 dates in maize (not shown). However, lack of correlation between conductance and  $D_0$  on a given date does not mean that microclimatic variation from place to place in the canopy did not cause variation in stomatal conductance, because the duration of fluctuations in leaf microclimate in relation to stomatal response times are not known. Sources of leaf-to-leaf variation in conductance other than  $D_0$  were not identified, but at least the minimally invasive system of measuring stomatal conductance did not automatically generate a negative correlation between conductance and  $D_0$ , as the photosynthesis system does in the mode of operation used here. The day-to-day variation in mean stomatal conductance, which correlated with  $D_0$ , thus cannot be attributed to an artifact of the measurement process, when using the minimally invasive system.

The minimally invasive system of measuring leaf and boundary layer conductances to water vapor worked well for fully exposed, nearly horizontal upper canopy leaves of these species under clear sky, open field conditions near mid-day. The method could be made non-invasive by using an infrared leaf temperature sensor, which could also average temperature over a larger area. However, at the moment, such sensors are not considered to be as accurate as thermocouples, and the accuracy of conductance measurements is strongly affected by the accuracy of leaf temperature measurements (Table 2). The relative accuracy of infrared thermometers and thermocouples in this application is uncertain. Measurements underneath canopies or under cloudy conditions would also require alteration of the radiation budget to account for different proportions of sky radiation. This method of measuring stomatal conductance is probably not well suited to general purpose measurements, particularly under variable cloud or canopy cover. However, under restricted sets of conditions it can provide information on the in situ



leaf environment and leaf boundary layer and stomatal conductances, which is difficult to obtain by other methods.

#### 4. Conclusions

An energy balance approach using temperatures of leaves and leaf replicas of the same size and orientation produced reasonably precise estimates of leaf boundary layer and stomatal conductances of horizontal upper canopy broad leaves under mid-day clear sky field conditions. This minimally invasive method agreed with estimates of stomatal conductance made with invasive systems, provided that  $D_0$  values were the same. The minimally invasive system had the advantage of both measuring the in situ  $D_0$  values and measuring stomatal conductances at those values. Use of the minimally invasive system of measuring stomatal conductance and  $D_0$  values in extensive maize and soybean canopies indicated that  $D_0$  varied sufficiently from day to day in this climate to result in about a two-fold variation in mid-day stomatal conductance in both species.

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